Structure and mechanism of an integral membrane enzyme, outer membrane phospholipase A from Escherichia coli

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Summary

All living organisms consist of cells, which are surrounded by membranes. These membranes are generally made up by phospholipids and membrane proteins. The membranes function as a barrier and allow cells to acquire a specific chemical environment, which is essential for the correct functioning of the organism. Yet, cells and organisms must be able to interact with their environment via the membranes. While the phospholipid bilayer fulfils the barrier function, the membrane proteins embedded in this bilayer perform specific tasks such as interaction with neighbouring cells and signal transduction. For a detailed understanding of these processes, structural knowledge of the membrane proteins is crucial. However, these proteins have eluded large-scale structural characterisation, and merely a handful have been characterised in detail.

This thesis describes the structure determination of the Outer Membrane Phospholipase A (OMPLA) from the outer membrane of the *Escherichia coli* bacterium. This integral membrane enzyme hydrolyses the phospholipids that compose the membrane. Due to this hydrolysis the membrane becomes more permeable.

The enzyme has a β-barrel fold with 12 antiparallel β-strands spanning the hydrophobic part of the membrane. The β-barrel architecture is a common motif for outer membrane proteins. The various classes of β-barrel membrane proteins are discussed in the first chapter. This chapter also reviews the current biochemical knowledge of OMPLA.

The activity of OMPLA is strictly regulated, because continuous phospholipid hydrolysis and the corresponding loss in membrane integrity are detrimental to the

bacterium. Reversible dimerisation is the major regulatory mechanism for the activity of OMPLA; the monomeric enzyme is inactive whereas the dimeric enzyme form is active. The second chapter reports the structure determination of both monomeric and dimeric OMPLA. These structures demonstrate that only in the dimer productive substrate binding pockets are formed, which explains why monomeric OMPLA is inactive.

However, dimerisation alone is not sufficient for catalysis. OMPLA also requires calcium binding for activity. Each active site in the dimeric complex binds one calcium ion. The positive charge of this ion is essential for stabilisation of the negatively charged intermediates and transition states that occur during the hydrolysis of phospholipids. In monomeric OMPLA also a calcium ion is bound, but at a location different from that in the dimeric enzyme. It is just outside the active site. Upon dimerisation of OMPLA this calcium could relocate to the active site, thus forming the catalytically competent complex. Structural details of calcium binding in OMPLA are discussed in chapter three.

The arrangement of the active site residues in the active site of OMPLA (His142, Ser144 and Asn156) resembles that of the classical serine-hydrolase triad. This classical catalytic triad is a well-known structural motif formed by a serine, a histidine and an aspartate residue that together can perform efficient hydrolysis of peptides and ester compounds. The active site of OMPLA, however, is unique as it contains an asparagine instead of an aspartate residue. The fourth chapter discusses the role of this asparagine residue in catalysis. The asparagine is not involved in the positioning of the histidine side-chain, nor contributes to thermostability. Instead, it most likely selects the correct tautomeric form of the active site histidine.

The focus in chapters five and six is shifted to the process of crystal growth of membrane proteins. A better understanding of the crystallogenesis process may enable successful structural investigations of many more membrane proteins. The fifth chapter presents the determination of the structure of the detergent in OMPLA crystals using neutron diffraction contrast variation experiments. The detergent is arranged around the hydrophobic surfaces of the β-barrel. It has formed a continuous three-dimensional phase throughout the crystal, which suggests that during crystallisation detergent micelles coalesce.

The sixth chapter reviews the crystallisation conditions of OMPLA. Based on these experiments recommendations are suggested for crystallisation of other membrane proteins. As we observed a striking difference between crystallisation behavior of hanging drop vapor diffusion experiments and micro-batch crystallisation under oil, it is strongly recommended to screen conditions using different methods.

Finally, the seventh chapter shortly reviews the impact of the structures on the biological understanding of OMPLA and gives perspectives for future research.